I-010541-P-0124

DEC 0 8 2006

U.S. Department of the Interior
Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Attention: David Erdahl, Ph.D.
Branch Chief, AADAP
4050 Bridger Canyon Road
Bozeman, MT 59715

Re: Isoeugenol (AQUI-S) target animal safety study on rainbow trout

Dear Dr. Erdahl:

The target animal safety section for the use of isoeugenol (AQUI-S) to sedate freshwater-reared finfish to handleable remains incomplete. We reviewed your submission dated April 18, 2006, as amended on May 8 and May 17, 2006, and find these data to be acceptable. This study demonstrates that there is an adequate margin of safety above 40 mg/L AQUI-S for sedation of rainbow trout to handleable. To complete the target animal safety technical section for all freshwater-reared salmonids, you will need one additional study in another salmonid species. To complete the technical sections for coolwater and warmwater species of freshwater-reared finfish, you will need acceptable studies in two representative species from each temperature group. The target animal safety technical section for the use of isoeugenol (AQUI-S) to sedate all species of freshwater-reared finfish will be complete upon acceptance of studies in two species from each temperature group, as agreed in our meeting on February 7, 2006, with AQUI-S, New Zealand, Ltd.

ADDITIONAL COMMENTS

During our review of your final study report, we noted the following items. While, ultimately, these items did not impact our ability to accept your data and conclusions, you should address them in future investigations.

1. Your cover letters identify the study number as AQUIS-01-EFF.2-1, whereas the final study report and data capture forms are consistently labeled AQUIS-03-RBT.2. In the future, please assign a unique study number to each study and include the study number in the Freedom of Information Summary.

2. In examining the training logs, we noted that the person performing necropsies did not read the SOP on necropsies until after he had performed all of the necropsies for the study. Please ensure that all study personnel have read the SOPs and MSDSs before the study begins.

3. You note in Deviation 3 that a few fish may have jumped out of their recovery tanks. In future studies, you should make modifications to ensure that fish cannot escape from the test tanks.

4. We note that a standard concentration above the highest test concentration was not used to generate the calibration curve for dose verification. In future studies, you should include a standard whose concentration is higher than the highest test exposure concentration.

5. The study procedure deviated from the study protocol in that only histopathological lesions that were moderate or severe and that appeared to be test article-induced prompted microscopic examination of the same tissue at the next longest exposure-duration of the same concentration. In future studies, please adhere to the protocol by examining tissues in the next lower dose group when any lesions are found. You may seek guidance from CVM on how to revise the protocol if you believe greater clarity is needed with respect to when tissues should be evaluated in order to distinguish potential test-article induced lesions from spontaneous disease and/or background lesions.

6. We note that you used fish from a single reference tank to determine the ET80s. In future studies, please include fish from all reference tanks when determining the ET80s.

7. When you randomized fish to tanks, you used the same randomization scheme for each round of allocation. When randomizing fish to tanks, please use a different randomization scheme for each round of allocation.

8. For mortality rates, you calculated the mean and 95% confidence interval of the mean by using a modified Freeman-Tukey double arcsine transformation, which is appropriate for proportions. The formula you used, $percent = [(\sin p')^2] * 100$, to translate the mean of the arcsines back into the original units of proportions was not correct. This is the inverse of the arcsine transformation. You should have used the inverse of the modified Freeman-Tukey transformation, which is:

$$percent = 0.5\{1 - sign(\cos(2t))[1 - (\sin(2t) + (\sin(2t) - 1/\sin(2t))/n)^2]^{0.5}\}.$$

However, since this is a sine transformation, sometimes the mean is not within the 95% confidence interval. Additionally, as there are only four observations per concentration and exposure time, computation of a confidence interval might not be useful. Instead, simply report either the values and/or indicate the range of values in future studies.

FOI SUMMARY COMMENTS

A revised FOI Summary section is enclosed. We revised your summary of the study primarily by removing the table of microscopic lesions, since no lesions were determined to be clinically significant, and by including tables describing the survival of fish and the indices of safety you derived. We believe the tables contain valuable information concerning expected survivability and time points at which isoeugenol (AQUI-S) may induce an unacceptable level of mortality

in a treated population. Using rainbow trout as a representative species, the information in the tables may hold significant inferential value for those using isoeugenol (AQUI-S) in other species of finfish not specifically tested.

If you submit correspondence relating to this letter, you should reference this letter by date and the principal submission identifier(s) found at the top of this letter. If you have any questions about this letter, please contact me at 301-827-7571, or Dr. Donald Prater, Leader, Aquaculture Drugs Team at 301-827-7567.

Sincerely,

Joan C. Gotthardt 12/7/06

Joan C. Gotthardt, D.V.M.
Director, Division of Therapeutic
Drugs for Food Animals
Office of New Animal Drug Evaluation
Center for Veterinary Medicine

Enclosure: FOI Summary

III. TARGET ANIMAL SAFETY:

A. Toxicity Study

Title: "The safety of AQUI-S as an Anesthetic on Rainbow Trout *Oncorhynchus mykiss*"

Study Director: James D. Bowker, MS

Study Location: U.S. Fish and Wildlife Service
Bozeman Fish Technology Center
4050 Bridger Canyon Road
Bozeman, MT 59715

General Study Design:

1. Purpose: To demonstrate the safety of a 20 to 40 mg/L dose range of isoeugenol (AQUI-S) administered as a static bath to small fingerling rainbow trout. This study was conducted in accordance with Good Laboratory Practice (21 CFR 58) regulations.

2. Animals: Small fingerling rainbow trout; total length ranged from 3.1 to 5.2 cm

3. Test article: 50% isoeugenol (AQUI-S)

4. Study Design: Groups of 20 test fish were exposed to 0, 20, 40 (1X), or 80 (2X) mg/L AQUI-S for one of four durations. Durations were selected to approximate 50 to 100% survival at each concentration (see Table 1). Each of the sixteen combinations of AQUI-S concentration and exposure duration was replicated four times.

   From a reference population, healthy fish were collected and examined to establish a baseline for fish health. The examinations included a gross morphologic and microscopic evaluation. A preliminary study was conducted on fish from the reference population to establish an estimated time for sedation to handleable at the 80th percentile, ET80. The ET80 values were then used to determine the exposure durations for each test concentration (Table 1). The exposure duration for the control group (0 mg/L) was set at the longest duration (20 mg/L).

   Fish were randomly allocated to test tanks prior to exposure to isoeugenol (AQUI-S). Following exposure, four fish (up to two dead fish and up to four live fish) per replicate were collected for gross morphologic and microscopic evaluation upon death or at 24 hours post-exposure (for live fish).

Table 1. Exposure durations for each concentration.

Concentration	*T1 (minutes)*	*T2 (minutes)*	*T3 (minutes)*	*T4 (minutes)*
0 mg/L	22.1	35.0	51.5	66.2
20 mg/L	22.1	35.0	51.5	66.2
40 mg/L	5.5	6.3	9.0	13.0
80 mg/L	1.9	2.1	2.7	3.0

5. Measurements and Observations: Mortality, gross morphologic, and microscopic lesions were the primary variables. Fish behavior, AQUI-S concentration, and water quality parameters were evaluated as secondary variables. Microscopic evaluation included the following tissues: skin, eye, gill, muscle, brain, heart, spleen, liver, anterior kidney, posterior kidney, stomach, pyloric intestine, and rectal intestine.

Results: Table 2 summarizes the mean survival for each concentration at the exposure durations listed in Table 1. Maximum exposure durations at 20, 40 and 80 mg/L AQUI-S where survival was acceptable (i.e., 95%) were approximately 22, 6, and 2 minutes, respectively (see Table 2). Safety breakpoint intervals (the exposure-duration range during which mean survival of test fish dropped below 95%) for each of the doses tested were 22 to 35, 6 to 9, and 2 to 3 minutes, respectively. And margins of safety (the time difference between the maximum exposure duration and the time it takes to sedate 80% of fish to handleable) for each concentration tested were 17, 4, and 1 minute, respectively (see Table 3).

Microscopic findings in treated fish included gill epithelial separation and hypertrophy, but were not considered clinically significant. At 40 mg/L, headshaking behavior occurred in 25 to 50% of the fish in all experimental units upon immersion for no more than 15 to 45 seconds; behavior during recovery was normal. At 80 mg/L, headshaking occurred in 75% of the fish in all experimental units except one; behavior during recovery was normal.

Table 2. Mean survival. Exposure durations T1, T2, T3, and T4 are specified in Table 1.

Survival	*T1*	*T2*	*T3*	*T4*
0 mg/L	99 %	100 %	99 %	100 %
20 mg/L	100 %	94 %	75 %	46 %
40 mg/L	99 %	100 %	83 %	68 %
80 mg/L	100 %	98 %	86 %	71 %

Table 3. Margin of safety based on longest exposure time and determined ET80, where the ET80 is the 80th percentile time-to-handleable.

AQUI-S concentration (mg/L)	*Longest exposure with no mortality (minutes)*	*ET80 (minutes)*	*Safety Margin (minutes)*
20	22.08	5.09	16.99
40	6.3	1.84	4.46
80	2.1	0.92	1.18

Conclusions: Isoeugenol (AQUI-S) is safe when administered to rainbow trout at doses ranging from 20 to 40 mg/L for sedation to handleable. There is an adequate margin of safety above 40 mg/L based on concentration and duration of exposure. The label for isoeugenol (AQUI-S) should include a statement indicating the drug should be tested on a small number of fish before administration to large groups and the lowest label dose should be used to achieve the desired level of sedation for a given population of fish. The label should also include a precaution that salmonids may exhibit temporary headshaking upon immersion.

United States Department of the Interior
FISH AND WILDLIFE SERVICE

AQUATIC ANIMAL DRUG APPROVAL PARTNERSHIP PROGRAM
4050 BRIDGER CANYON ROAD
BOZEMAN, MT 59715
(406) 587-9265/FAX 582-0242

April 18, 2006

Dr. Joan Gotthardt
Director, Division of Therapeutic Drugs
for Food Animals
Document Control Unit, HFV-199
Center for Veterinary Medicine
7500 Standish Place, MPN-2
Rockville, MD 20855

Dear Dr. Gotthardt:

The purpose of this submission is to request a formal review of the enclosed Final Study Report (FSR) titled "The Safety of AQUI-S® as an Anesthetic on Rainbow Trout *Oncorhynchus mykiss.*" The FSR is identified by Study Number AQUIS-01-EFF.2-1. Please note that we also request that the FSR be included in the AQUI-S® target animal safety technical section in support of a New Animal Drug Approval for AQUI-S®, and that the FSR be filed in the Service's Investigational New Animal Drug (INAD) file #10-541. We refer to your file number INAD 10-541 P-0084 dated July 01, 2005.

The enclosed FSR summarizes results from a study in which the maximum safe durations (survival ≥ 95%), safety breakpoint intervals, and the margins of safety were established for 20, 40 and 80 mg/L AQUI-S® under the study conditions specified in the FSR. Specifically, the FSR demonstrated the following: 1) The maximum safe durations for 20, 40, and 80 mg/L AQUI-S® were approximately 22, 6, and 2 min respectively, 2) the safety breakpoint intervals for 20, 40, and 80 mg/L AQUI-S® were approximately 22 - 35, 6 - 9, and 2 - 3 min, respectively; 3) the margins of safety (maximum safe duration minus the ET80 for that dose) for 20, 40, and 80 mg/L AQUI-S® were 17, 4, and 1 min, respectively; and 4) the only pathologies of note that were observed were gill epithelial separation and gill hypertrophy. Although the noted pathologies could have been test-article induced and potential safety concerns, based on documentation in the literature it is likely that these morphological changes in gill tissue were procedural artifacts of histological preparation. It is also important to note that severe pathologies were only noted at relatively low prevalence, and without an easily discernable "pattern of effect" between treatments. Hence, it is our contention that at the "safe" AQUI-S® dosages these histological changes were not pathologic, nor did they contribute to test fish mortality. We speculate that test

fish that died during the study did so because they simply became too deeply sedated (via a combination of dose and duration) to recover. This result/conclusion was certainly not unexpected. However, it is our opinion that the target animal safety data generated in this study support the approval of AQUI-S® for use at 20 and 40 mg/L to sedate all freshwater salmonid fishes for management and handleable purpose. Please note that a draft Freedom of Information (FOI) summary of this study is appended to the FSR.

The current sponsor of INAD #10-541 is Dr. David Erdahl, U.S. Fish and Wildlife Service, Branch Chief - AADAP Program, 4050 Bridger Canyon Road, Bozeman, MT 59715. We would like to thank you in advance for your time and consideration with respect to the above-described request. If you have questions, please contact Dr. Erdahl at (406) 994-9904.

Sincerely,

Dr. David Erdahl
Branch Chief - AADAP Program

enclosures: FSR titled "The Safety of AQUI-S® as an Anesthetic on Rainbow Trout *Oncorhynchus mykiss.*"
Draft TAS FOI

Date of Approval _____

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA xxx-xxx

(proprietary and established product name)

"For sedation of all freshwater fishes for management and handling purposes."

Sponsored by:

(Sponsor with U.S. address)

1. GENERAL INFORMATION

a.	File Number:	NADA xxx-xxx
b.	Sponsor:	(Company Name)
c.	Drug Labeler Code:	xxxxxx
d.	Established Name:	AQUI-S®
e.	Proprietary Name:	(Product name)
f.	How Supplied:	100 or 1,000 mL plastic bottle
g.	How Dispensed:	Over the counter
h.	Amount of Active Ingredient:	50%
i.	Route of Administration:	Static bath
j.	Species/class:	All freshwater fishes
k.	Recommended Dosage:	20 - 40 mg/L until sedated for management and handling procedures
l.	Pharmacological Category:	Anesthetic
m.	Indications:	For sedation of all freshwater fishes for management and handling procedures

2. EFFECTIVENESS:

A. Substantial Evidence

1) Study Number xxxx-xxxxx
2) Study Number xxxx-xxxxx
3) etc.

3. TARGET ANIMAL SAFETY

A. Target Animal Safety Studies:

Title: "The Safety of AQUI-S® as an Anesthetic on Rainbow Trout *Oncorhynchus mykiss*"

Study Director: James D. Bowker, MS

Study Location: U. S. Fish and Wildlife Service
Bozeman Fish Technology Center
4050 Bridger Canyon Road
Bozeman, MT 59715

General Design of the Study:

A. Purpose: To demonstrate the safety of 20, 40, and 80 mg/L AQUI-S® administered as a static bath to small fingerling rainbow trout *Oncorhynchus mykiss*.

B. Animals: Small fingerling rainbow trout

C. Test Article: AQUI-S®

D. Study Design: Groups of 20 test fish were exposed to 0, 20, 40 (1X) or 80 (2X) mg/L AQUI-S® for one of four durations. Durations were determined to achieve 50 - 100% survival. Each of the 16 combinations of AQUI-S® concentration and exposure duration was replicated four times. "Healthy-appearing" fish were collected to evaluate baseline fish health and histological condition. No more than four fish (up to two dead fish and up to four live fish) were collected for gross examination and histology upon death or at 24-hr post-exposure (for live fish). For each histologic criterion evaluated, the histologist ranked the observed changes

as 1 = none, 2 = mild, 3 = moderate, or 4 = severe. The following histologic criteria were evaluated.

Eye	Epithelial thickness Degeneration	Edema
Gill	Scattered fusion Epithelial Separation Basal Hyperplasia	Aneurysms in gill capilaries Hypertrophy of gill epithelium Epithelial necrosis
Posterior Kidney	Tubule necrosis Hyaline droplet degeneration Hydropic degeneration	Tubule Swelling Hematopoietic Hyperplasia
Skin	Mucous cell number Necrosis	Degeneration
Brain	Fluid in Vessicles Congestion	Degeneration
Heart	Congestion	Degeneration
Muscle	Edema	Degeneration of muscle fibers
Stomach	Degeneration of mucousal epithelium	Congestion
Pyloric Intestine	Degeneration of mucousal epithelium	Congestion
Rectal Intestine	Degeneration of mucousal epithelium	Congestion
Spleen	Congestion Degeneration	Reduction in lymphoid tissue
Liver	Cytoplasmic vacuoloation of hepatocytes Diffuse necrosis of hepatocytes	Nuclear vacuoloation of hepatocytes Focal necorsis of hepatocytes
Anterior Kidney	Hematopoietic hyperplasia Degeneration	Congestion

E. Parameters measured: Mortality, gross pathology, histopathology criteria, behavior, AQUI-S® concentration, and water quality parameters.

GLP Compliant: Yes

Results: Maximum exposure durations at 20, 40 (1X) and 80 (2X) mg/L AQUI-S® where survival was acceptable (i.e., ≥ 95%) were approximately 22, 6, and 2 min, respectively. Safety breakpoint intervals for each of the doses tested were 22 - 35, 6 - 9, and 2 - 3 min, respectively. And margins of safety for each concentration

tested were 17, 4, and 1 min, respectively. The only histological changes observed that could be considered test-article induced and potentially pathologic were gill epithelial separation and gill hypertrophy. However, these gill morphological changes could also have been procedural artifacts. Regardless, the gill epithelial separation and gill hypertrophy observed in this study were not considered severe because mean percent survival at maximum safe exposure durations identified was very high (≥ 98%).

Conclusions: An acceptable margin of safety exists for the 1X and 2X dose groups. Use of AQUI-S® to sedate rainbow trout for management and handling purposes is safe. However, ensure that you sedate fish to the appropriate end-point, and test a small batch of fish before use on a production scale.

4. **HUMAN SAFETY:**

5. **AGENCY CONCLUSIONS:**

6. **ATTACHMENTS:**

(facsimile labeling)